On page 33, line 14, after "Madxho-reverse" insert --(SEQ ID NO:14)--.

On page 38, line 1, after "Ma524PCR-1" insert --(SEQ ID NO:15)--.

On page 38, line 5, after "Ma524PCR-2" insert --(SEQ ID NO:16)--.

On page 38, line 15, after "Δ6 desaturase" insert --(SEQ ID NO:17 and SEQ ID NO:18)--.

On page 38, line 16, after "Δ12 desaturase" insert --(SEQ ID NO:19 and SEQ ID NO:20)--.

On page 39, line 2, after "Ma648PCR-for" insert --(SEQ ID NO:21)--.

On page 39, line 4, after "Ma648PCR-for" insert --(SEQ ID NO:22)--.

Please insert the attached paper readable Sequence Listing as pages 53-67 of the attached substitute specification and renumber the remaining pages accordingly.

produced by binding of target or probe, respectively, as may be done with the BIAcore system.

The invention will be better understood by reference to the following non-limiting examples.

Examples

Example 1

Expression of ω-3 desaturase from *C. elegans* in transgenic plants.

The Δ15/ω-3 activity of *Brassica napus* can be increased by the expression of an ω-3 desaturase from *C. elegans*. The fat-1 cDNA clone (Genbank accession L41807; Spychalla, J. P., Kinney, A. J., and Browse, J. 1997 P.N.A.S. 94, 1142-1147, SEQ ID NO:1 and SEQ ID NO:2) was obtained from John Browse at Washington State University. The fat-1 cDNA was modified by PCR to introduce cloning sites using the following primers:

Fat-1forward (SEQ ID NO:3):

5'-CUACUACUACUACTGCAGACAATGGTCGCTCATTCCTCAGA-3'

Fat-1reverse (SEQ ID NO:4):

5'- CAUCAUCAUCAUGCGGCCGCTTACTTGGCCTTTGCCTT - 3'

These primers allowed the amplification of the entire coding region and added PstI and NotI sites to the 5'- and 3'-ends, respectively. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5562. The sequence was verified by sequencing of both strands to be sure no changes were introduced by PCR.

A once base pair difference was observed in the fat-1 coding region from pCGN5562 vs. the fat-1 Genbank sequence. The C at position 705 of the fat-1 sequence was changed to

an A in pCGN5562. This creates a change of a GAC codon to GAA, changing the Asp residue at position 231 of fat-1 to a Glu residue. This identical change was observed in products of two independent PCR reactions using fat-1 template and most likely is not a result of PCR mis-incorporation of a nucleotide. For seed specific expression, the Fat-1 coding region was cut out of pCGN5562 as a PstI/NotI fragment and inserted between the PstI/NotI sites of the binary vector, pCGN8623, to create pCGN5563. PCGN5563 can be introduced into *Brassica napus* via *Agrobacterium*-mediated transformation.

Construction of pCGN8623

The polylinker region of the napin promoter cassette, pCGN7770, was replaced by ligating the following oligonucleotides:

5'- TCGACCTGCAGGAAGCTTGCGGCCGCGGATCC -3' (SEQ ID NO:5) and

5'- TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGG-3' (SEQ ID NO:6). These oligonucleotides were ligated into SalI/XhoI-digested pCGN7770 to produce pCGN8619. These oligos encode BamHI, NotI, HindIII, and PstI restriction sites. pCGN8619 contains the oligos oriented such that the PstI site is closest to the napin 5' regulatory region. A fragment containing the napin 5' regulatory region, polylinker, and napin 3' region was removed from pCGN8619 by digestion with Asp718I. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8623.

To produce high levels of stearidonic acid in *Brassica*, the *C. elegans* ω-3 desaturase can be combined with Δ6- and Δ12-desaturases from *Mortierella alpina*. PCGN5563-

transformed plants may be crossed with pCGN5544-transformed plants expressing the Δ6-and Δ12-desaturases, described below.

The resulting F1 seeds can be analyzed for stearidonic acid content and selected F1 plants can be used for self-pollination to produce F2 seed, or as donors for production of dihaploids, or additional crosses.

An alternative method to combine the fat-1 cDNA with *M. alpina* Δ6 and Δ12 desaturases is to combine them on one T-DNA for transformation. The fat-1 coding region from pCGN5562 can be cut out as a PstI/NotI fragment and inserted into PstI/NotI digested pCGN8619. The transcriptional unit consisting of the napin 5' regulatory region, the fat-1 coding region, and the napin 3'-regulatory region can be cut out as a Sse8387I fragment and inserted into pCGN5544 cut with Sse8387I. The resulting plasmid would contain three napin transcriptional units containing the *C. elegans* ω-3 desaturase, *M. alpina* Δ6 desaturase, and *M. alpina* Δ12 desaturase, all oriented in the same direction as the 35S/nptII/tml transcriptional unit used for selection of transformed tissue.

Example 2

Over-Expression of Δ15-desaturase Activity in Transgenic Canola

The Δ15-desaturase activity of *Brassica napus* can be increased by over-expression of the Δ15-desaturase cDNA clone.

A *B. napus* Δ15-desaturase cDNA clone was obtained by PCR amplification of first-strand cDNA derived from *B. napus* cv. 212/86. The primers were based on published sequence: Genbank # L01418 Arondel et al, 1992 Science 258:1353-1355 (SEQ ID NO:7 and SEQ ID NO:8).

The following primers were used:

Bnd15-FORWARD (SEQ ID NO:9)

5'-CUACUACUACUAGAGCTCAGCGATGGTTGTTGCTATGGAC-3'

Bnd15-REVERSE (SEQ ID NO:10)

5'-CAUCAUCAUCAUGAATTCTTAATTGATTTTAGATTTG-3'

These primers allowed the amplification of the entire coding region and added SacI and EcoRI sites to the 5'- and 3'-ends, respectively

The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5520. The sequence was verified by sequencing of both strands to be sure that the open reading frame remained intact. For seed specific expression, the Δ15-desaturase coding region was cut out of pCGN5520 as a BamHI/SalI fragment and inserted between the BglII and XhoI sites of the pCGN7770, to create pCGN5557. The PstI fragment of pCGN5557 containing the napin 5'-regulatory region, *B. napus* Δ15-desaturase, and napin 3'-regulatory region was inserted into the PstI site of the binary vector, pCGN5138 to produce pCGN5558. pCGN5558 was introduced into *Brassica napus* via *Agrobacterium*-mediated transformation.

To produce high levels of stearidonic acid in *Brassica*, the Δ15-desaturase can be combined with Δ6- and Δ12-desaturases from *Mortierella alpina*. PCGN5558-transformed plants may be crossed with pCGN5544-transformed plants expressing the Δ6 and Δ12-desaturases. The resulting F1 seeds are analyzed for stearidonic acid content. GC-FAME analysis of F1 half-seeds revealed a significant accumulation of SDA in the seed oil of the *Brassica* lines. SDA levels (18:4) of greater than approximately 25% were obtained in hemizygous lines and are provided in Table 1. Selected F1 plants can be used for self-pollination to produce F2 seed, or as donors for production of dihaploids, or additional crosses.

Example 3

Expression of Δ5 Desaturase in Plants Expression in Leaves

Ma29 is a putative *M. alpina* Δ5 desaturase as determined by sequence homology (SEQ ID NO:11 and SEQ ID NO:12). This experiment was designed to determine whether leaves expressing Ma29 (as determined by Northern) were able to convert exogenously applied DGLA (20:3) to ARA (20:4).

The Ma29 desaturase cDNA was modified by PCR to introduce convenient restriction sites for cloning. The desaturase coding region has been inserted into a d35 cassette under the control of the double 35S promoter for expression in *Brassica* leaves (pCGN5525) following standard protocols (*see* USPN 5,424,200 and USPN 5,106,739). Transgenic *Brassica* plants containing pCGN5525 were generated following standard protocols (*see* USPN 5,188,958 and USPN 5,463,174).

In the first experiment, three plants were used: a control, LPOO4-1, and two transgenics, 5525-23 and 5525-29. LP004 is a low-linolenic *Brassica* variety. Leaves of each were selected for one of three treatments: water, GLA or DGLA. GLA and DGLA were purchased as sodium salts from NuChek Prep and dissolved in water at 1 mg/ml. Aliquots were capped under N_2 and stored at -70 degrees C. Leaves were treated by applying a 50 µl drop to the upper surface and gently spreading with a gloved finger to cover the entire surface. Applications were made approximately 30 minutes before the end of the light cycle to minimize any photo-oxidation of the applied fatty acids. After 6 days of treatment one leaf from each treatment was harvested and cut in half through the mid rib. One half was washed with water to attempt to remove unincorporated fatty acid. Leaf samples were lyophilized overnight, and fatty acid composition determined by gas chromatography (GC). The results are shown in Table 3.

Leaves treated with GLA contained from 1.56 to 2.4 wt% GLA. The fatty acid analysis showed that the lipid composition of control and transgenic leaves was essentially the same. Leaves of control plants treated with DGLA contained 1.2-1.9 w% DGLA and background amounts of ARA (.26-.27 wt%). Transgenic leaves contained only .2-.7 wt% DGLA, but levels of ARA were increased (.74-1.1 wt%) indicating that the DGLA was converted to ARA in these leaves.

Expression in Seed

The purpose of this experiment was to determine whether a construct with the seed specific napin promoter would enable expression in seed.

The Ma29 cDNA was modified by PCR to introduce *Xho*I cloning sites upstream and downstream of the start and stop codons, respectively, using the following primers:

Madxho-forward (SEQ ID NO:13):

5'-CUACUACUACUACTCGAGCAAGATGGGAACGGACCAAGG

Madxho-reverse (SEQ ID NO:14):

5'-CAUCAUCAUCAUCTCGAGCTACTCTTCCTTGGGACGGAG

The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5522 and the Δ5 desaturase sequence was verified by sequencing of both strands.

For seed-specific expression, the Ma29 coding region was cut out of pCGN5522 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5528. The *Hin*dIII fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the *Hin*dIII site of pCGN1557 to create pCGN5531. Two copies of the napin transcriptional unit were inserted in tandem. This tandem construct can permit higher expression of the

Ma524PCR-1 (SEQ ID NO:15)

5'-CUACUACUACUATCTAGACTCGAGACCATGGCTGCTGCT
CCAGTGTG

Ma524PCR-2 (SEQ ID NO:16)

5'-CAUCAUCAUCAUAGGCCTCGAGTTACTGCGCCTTACCCAT

These primers allowed the amplification of the entire coding region and added *Xba*I and *Xho*I sites to the 5'-end and *Xho*I and *Stu*I sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5535 and the Δ6 desaturase sequence was verified by sequencing of both strands.

Construction of pCGN5544

Plant expression constructs were prepared to express the *Mortierella alpina* Δ6 desaturase and the *Mortierella alpina* Δ12 desaturase in a plant host cell. The constructs prepared utilized transcriptional initiation regions derived from genes preferentially expressed in a plant seed. Isolation of the cDNA sequences encoding the *M. alpina* Δ6 desaturase (SEQ ID NO:17 and SEQ ID NO:18) and *M. alpina* Δ12 desaturase (SEQ ID NO:19 and SEQ ID NO:20) are described in PCT Publications WO 98/46763 and WO 98/46764, the entireties of which are incorporated herein by reference.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5536. The *Not*I fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the *Not*I site of pCGN1557 to create pCGN5538.

The 5542 cDNA, encoding the *M. alpina* Δ12 desaturase, was modified by PCR to introduce cloning sites using the following primers:

Ma648PCR-for (SEQ ID NO:21)

5'-CUACUACUACUAGGATCCATGGCACCTCCCAACACT

Ma648PCR-for (SEQ ID NO:22)

5'-CAUCAUCAUCAUGGTACCTCGAGTTACTTCTTGAAAAAGAC

These primers allowed the amplification of the entire coding region and added a BamHI site to the 5' end and KpnI and XhoI sites to the 3' end. The PCR product was subcloned into pAMP1 (Gibco-BRL, Gaithersburg, MD) using the CloneAmp system (Gibco-BRL) to create pCGN5540, and the Δ12 desaturase sequence was verified by sequencing of both strands.

A seed preferential expression construct was prepared for the Δ12 desaturase cDNA sequence. The Ma648 coding region was cut out of pCGN5540 as a BamHI/XhoI fragment and inserted between the BglII and XhoI sites of the napin expression cassette, pCGN3223 (described in USPN 5,639,790), to create pCGN5542.

In order to express the *M. alpina* Δ6 and Δ12 desaturase sequences from the same T-DNA, the following construct for seed-preferential expression was prepared.

The NotI fragment of pCGN5536 containing the napin 5' transcriptional initiation region , the Ma524 coding region, and the napin 3' transcriptional termination region was inserted into the NotI site of pCGN5542 to create pCGN5544. The expression cassettes were oriented in such a way that the direction of transcription from Ma524 and Ma648 and the nptII marker is the same.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5536. The *Not*I fragment of pCGN5536 containing the napin 5' regulatory